HHS Public Access

Author manuscript

Clin Cancer Res. Author manuscript; available in PMC 2017 August 30.

Published in final edited form as:

Clin Cancer Res. 2016 August 15; 22(16): 4039–4044. doi:10.1158/1078-0432.CCR-15-2366.

Impact of genes highly correlated with *MMSET* myeloma on survival of Non-*MMSET* myeloma patients

S. Peter Wu[1], **Ruth M. Pfeiffer**[2], **Inhye E. Ahn**[1], **Sham Mailankody**[3], **Pieter Sonneveld**[4], **Mark van Duin**[4], **Nikhil Munshi**[5], **Brian Walker**[6], **Gareth Morgan**[7], and **Ola Landgren**[3]

[1]Multiple Myeloma Section, National Cancer Institute, National Institutes of Health, Bethesda, MD, USA [2]Department of Cancer Epidemiology and Genetics, Biostatistics Branch, National Cancer Institute, National Institutes of Health, Rockville, MD, USA [3]Myeloma Service, Memorial Sloan-Kettering Cancer Center, New York, NY, USA [4]Department of Hematology, Erasmus University Medical Center, Rotterdam, Netherlands [5]Lebow Institute of Myeloma Therapeutics and Jerome Lipper Multiple Myeloma Center, Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA, USA [6]Section of Haemato-Oncology, The Institute of Cancer Research, London, UK [7]Myeloma Institute for Research and Therapy, University of Arkansas for Medical Sciences, Little Rock, AR, USA

Abstract

Purpose—The poor prognosis of multiple myeloma with t(4;14) is driven by the fusion of genes encoding *MMSET* and immunoglobulin heavy chain. Specific genes affected by *MMSET* and their clinical implications in Non-*MMSET* myeloma remain undetermined.

Experimental design—We obtained gene-expression profiles of 1,032 newly diagnosed myeloma patients enrolled in Total Therapy 2, Total Therapy 3, Myeloma IX, and HOVON65-GMMGHD4 trials, and 156 patients from Multiple Myeloma Resource Collection. Probes most correlated with *MMSET* myeloma were selected based on a multivariable linear regression and Bonferroni correction, and refined based on the strength of association with survival in Non-*MMSET* patients.

Results—Ten *MMSET*-like probes were associated with poor survival in Non-*MMSET* myeloma. Non-*MMSET* myeloma patients in the highest quartile of the 10-gene signature (*MMSET*-like myeloma) had 5-year overall survival similar to that of *MMSET* myeloma (highest quartile vs. lowest quartile hazard ratio [HR]=2.0, 95% confidence interval [CI] 1.5-2.8 in

Corresponding author: Ola Landgren, Chief of Myeloma Service, Memorial Sloan Kettering Cancer Center, 1275 York Ave, New York, NY 10065, USA; office 212-639-5153; landgrec@mskcc.org.

Disclosure of Potential Conflict of Interest: Dr. Landgren has given scientific presentations at meeting funded by Onyx Pharmaceuticals/AMGEN, Celgene, BMS and Jansen; has served on the Independent Data Monitoring Committee for clinical trials by Millennium Pharmaceuticals/Takeda; and has served on the advisory committee for Medscape Educations, Myeloma program.

Authors' Contributions: Contribution: S.P.W, R.M.P., and O.L. designed the study and performed bioinformatics analysis; S.P.W, R.M.P., I.E.A., S.M., P.S., M.D., N.M., B.W., and O.L. reviewed data analysis and wrote manuscript.

Supplementary information contains 3 tables and 1 figure, which are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

MMSET-like myeloma; HR=2.3, 95% CI: 1.6-3.3 in *MMSET* myeloma). Analyses of *MMSET*-like gene signature suggested the involvement of p53 and *MYC* pathways.

Conclusion—*MMSET*-like gene signature captures a subset of high-risk myeloma patients under-represented by conventional risk stratification platforms, and defines a distinct biological subtype.

Keywords

multiple myeloma; high risk myeloma; *MMSET*; t(4;14); cDNA microarray

Introduction

Multiple myeloma has extremely heterogeneous outcomes. Among many prognostic factors utilized in myeloma, translocation t(4;14)(p16.3;q32.3) is an oncogenic event associated with poor prognosis. (1) The key molecular target of t(4;14) is multiple myeloma SET domain (*MMSET*) at chromosomal band 4p16.3. (2-5) The detection of *MMSET* overexpression with gene-expression profiling (GEP) consistently identifies a high-risk subgroup in multiple myeloma. (6) While the prognostic significance of *MMSET* is well established, the underlying mechanism of its excess risk is poorly understood. Given *MMSET* encodes histone methyltransferase, its overexpression has been attributed to alter epigenetic regulation of genes involved in cell cycle progression and DNA damage repair. (7) However, downstream gene targets and molecular pathways regulated by *MMSET* remain unclear.

What is also unknown in myeloma is the presence of biological homology shared between high-risk and non-high-risk subgroups. This question comes within the context of the recent advancement of genetic sequencing, which identified diverse spectrum of disease biology that, at times, redefined conventional risk stratification and management. For instance, “BRCA-ness” was identified in up to 14% of non-small cell lung cancer and 15% of head and neck cancer patients due to epigenetic inactivation of genes responsible for DNA damage repair, such as *BRCA1* and *FNACF*. (8) In breast and ovarian cancers, next-generation sequencing demonstrated the presence of certain genes beyond *BRCA1/2*, such as *PALB2, ATM* or *CHEK2,* was strongly associated with an increased risk of cancer diagnosis and early death. (9-11) Recent discoveries in solid tumor suggest a substantial proportion of cancer patients harbors molecular signatures similar to those of high-risk subtypes.

We hypothesize there is an overlap of disease biology between the established high-risk myeloma and its non-high-risk counterpart. Specifically, the same genes involved in the pathogenesis and adverse outcomes of *MMSET* myeloma (6) could also be relevant to a subset of Non-*MMSET* patients with poor clinical outcomes (hereby refer to “*MMSET*-like myeloma”). To characterize genes and molecular pathways influencing survival across different myeloma subtypes, we assessed expression levels of 54,675 genes in 1,188 newly diagnosed multiple myeloma patients. Among 71 genes significantly altered in *MMSET* myeloma, 10 genes most strongly associated with survival were selected and combined into a GEP risk score. Patients who did not have detectable *MMSET* but were at the top quartile

of the 10-gene risk score were categorized as *MMSET*-like myeloma. Five-year survivals were similar between patients with *MMSET* myeloma and *MMSET*-like myeloma. Pathway analysis identified *MYC* and *TP53* transcriptional regulators as lead candidates targeted by the observed genes within the risk score. Our findings suggest there is a homology of aggressive disease biology and clinical outcomes shared between *MMSET* myeloma and a subset of non-*MMSET* myeloma.

Methods

Study design

From the NCBI Gene Expression Omnibus (GEO), we downloaded unprocessed CEL files from the following datasets: Total Therapy (TT) 2 (N=345, accession number GSE2658, NCT00083551); TT 3 (N=214, accession number GSE2658, NCT00081939); HOVON65/GMMG-HD4, (N=320, accession number GSE19784, ISRCTN64455289); Myeloma IX (N=247, accession number GSE15695, ISRCTN68454111); and Multiple Myeloma Reference Collection (MMRC) (N=288, accession number GSE26760). The sample size of each data set was determined after excluding 8 profiles (accession number GSE19784) that were normal plasma cells and 16 patients (accession number GSE26760) who were smoldering myeloma (n=11), MGUS (n=2), or plasma cell leukemia (n=3). Anonymized patient characteristics of TT trials were obtained from GEO and were identified with the same accession numbers. Anonymized patient characteristics of Myeloma IX and HOVON65/GMMG-HD4 trials were obtained through personal correspondence with Mark van Duin and Ping Wu, respectively. Anonymized patient characteristics of MMRC were obtained from Multiple Myeloma Genome Portal. (12) Selected characteristics of patients from the five studies are shown in Table 1.

All gene-expression data were derived from CD138+ purified plasma cells of newly diagnosed myeloma patients, which were hybridized to Affymetrix Human Genome U133 Plus 2.0 cDNA microarray (Santa Clara, CA). All raw CEL files were processed using the *justMAS* function in the *R* statistical programming language, and gene-expression levels were log2 transformed. The final dataset included GEPs of 1,188 myeloma patients with complete data for age, sex, beta-2 microglobulin, and albumin. For the HOVON65/GMMG-HD4 trial, FISH data regarding *MMSET* status was available for 241 patients; *MMSET* status by FISH versus gene expression revealed a correlation of 0.81 (Spearman's rho). For the analysis of survival outcomes, we excluded 156 patients from MMRC as it was not a clinical trial, and only used the remaining data from 1,032 patients.

Institutional Review Boards of respective institutions approved all studies. All subjects provided written informed consents approving the use of their samples for research purposes.

Statistical analysis

MMSET myeloma patients, non-MMSET myeloma patients, and genes associated with MMSET myeloma—To classify patients into *MMSET* or Non-*MMSET* myeloma, we used the previously reported microarray model using 700 gene probes to

assign subjects into one of seven molecular subtypes. (13) We assessed the association of *MMSET* myeloma with individual expression levels of 54,675 available probes. By using linear regression models for each probe and for each study, gene-expression levels were dependent variables of *MMSET* status, age (divided by 50 years or less, 51-60 years, 61-70 years, 71 years or older), sex, and International Staging System (ISS) stage. (14) For each probe, study-specific linear regression coefficients for *MMSET* myeloma were then combined across studies using a random effects meta-analysis. (15) Prior to finalizing the probes that were significantly associated with *MMSET* myeloma, all 700 gene probes used in the Arkansas model (13) were removed. We performed a random effects meta-analysis after Bonferroni correction for multiple testing ($p<0.05/54{,}675=9.14\times10^{-7}$). The absolute value of the random effects slope parameter for *MMSET* myeloma was 2 or greater, indicating *MMSET* myeloma had 2-fold or greater changes in log expression of a given gene.

Identification of probes associated with survival in Non-MMSET patients—To identify probes relevant to survival of non-*MMSET* patients, results from the aforementioned meta-analysis were analyzed by a stepwise variable selection (proc *phreg, SAS 9.3)* in a Cox proportional hazards model. Duration of follow-up was defined by the start of treatment until death or censoring. Censoring occurred when a subject reached 5 years or was lost to follow up. For the initial selection of probes, we included probes that passed Bonferroni correction for multiple testing, and those showed log 2 or greater changes of expression. For each probe, a minimal $p<0.1$ in a marginal Cox proportional hazards model was set for initial inclusion, and a criteria of $p<0.05$ was set to retain a probe in the model. All models were adjusted for age, sex, ISS stage (14) and treatment. (16-18) The risk score was calculated based on the adjusted Cox regression model (Appendix 1).

Validation—To assess the unbiased association of the risk score and survival, we conducted a 5-fold cross-validation. (18) Briefly, the original dataset was divided into five equal parts, with equal numbers of patients from individual studies in each part. Four of the five parts were used to develop a gene signature following the aforementioned procedures (training set). The remaining fifth part was used to compute the association of the risk score and survival using Cox regression models (test set). Validation was performed five times with each part serving as a test set once. Risk scores from five test sets were median-centered and combined to form an independently scored measure of risk.

Sensitivity analysis—To assess stability of our results, we conducted three separate sensitivity analyses (Supplemental Tables 1 and 2). First, we used survival outcomes throughout the full follow-up time of up to 98 months instead of censoring at 60 months. Second, we excluded patients who were treated with proteasome inhibitor-based regimens, such as VTD-PACE and PAD, from the analysis. Third, patients on Myeloma IX trial were coded separately if they encountered death or censoring before the second randomization for thalidomide maintenance. In all three sensitivity analyses, the main results remained unchanged.

Pathway analysis—To determine biological functions of the identified gene probes, pathway analysis was performed using Ingenuity Pathway Analysis software package and the molecular signaling database from the Broad Institute (MsigDB). (19) Gene networks were constructed using the upstream regulator analysis to identify transcription factors with the most interactions with selected genes (Figure 1).

Results

Among 1,032 myeloma patients included in this study, 139 (13.4%) had *MMSET* myeloma defined by GEP (Table 1). (13) In *MMSET* myeloma, the median age was 59 years (range 24-89), and 68% were males. Distributions of ISS stage I, II, and III were 48%, 30% and 22%, respectively. Similar to prior reports (6), *MMSET* myeloma was associated with a higher mortality after adjusting for age, sex, ISS stage and treatment (hazard ratio (HR) =1.7, $p<0.001$).

To determine if the same genes involved in *MMSET* myeloma were also relevant to survival of Non-*MMSET* myeloma patients, we took the following analytical approach: First, as described in the Methods, we obtained GEPs of 1,188 newly diagnosed myeloma patients and defined 71 gene probes correlated with *MMSET* myeloma (Supplemental Table 3). From these probes, we further identified those associated with 5-year survival in Non-*MMSET* patients and created a 10-gene risk score predictive of survival. Lastly, we conducted a functional pathway analysis.

Gene probes correlated with MMSET myeloma

After the random effects meta-analysis, we identified 71 gene probes (0.13%) correlated with *MMSET* myeloma. The selected genes showed 2-fold or greater changes in log-expressions (range 2.0 to 3.7 or -2.0 to -3.7) in *MMSET* patients compared to Non-*MMSET* patients, and meta-analytic *p*-values ranged from 1.9×10^{-11} to 5.2×10^{-36} (Supplemental Table 3). Genes highly correlated with *MMSET* myeloma included cyclin D1 (*CCND1*), cyclin D2 (*CCND2*), a transcription factor Kruppel-like factor 4 (*KLF4*), ubiquitin carboxyl-terminal esterase L1 (*UCHL1),* and alpha-2-glycoprotein (*AZGP1*) (Supplemental Table 3).

Probes enriched in MMSET myeloma, Non-MMSET myeloma, and survival

From the identified 71 gene probes, 10 genes were strongly associated with 5-year survival of Non-*MMSET* patients (Table 2). *AZGP1* and *CCND1* were most significantly associated with survival (probe-specific HRs: 0.89-0.91 for *CCND1* and 1.07-1.14 for *AZGP1*, $p<0.001$). To define risk scores relevant to survival of non-*MMSET* myeloma patients, a multivariable Cox proportional hazards model was applied to the 10 genes. Risk score groups of the first quartile (low-risk) and the fourth quartile (high-risk) were compared within Non-*MMSET* patients in cross-validation. High-risk Non-*MMSET* patients (here by referred as "*MMSET*-like myeloma") had a similarly increased risk of mortality (HR=2.0, 95% confidence interval (CI) 1.5-2.8, $p<0.001$) comparable to *MMSET* patients (HR=2.3, 95% CI 1.6-3.3, $p<0.001$).

Pathway analysis

To characterize genes and molecular pathways influencing survival across different myeloma subtypes, we conducted analysis of the 10 genes associated with 5-year survival in *MMSET*-like myeloma by using IPA (Ingenuity® Systems). Pathway analysis identified *MYC* and *TP53* transcriptional regulators as lead candidates for the observed gene expression changes within the gene signature risk score (Figure 1). *TP53* was identified as a transcriptional regulator of four genes (*CCND1, PTP4A3, MYBL1,* and *ROBO1*) ($p=1.9\times10^{-3}$), and *MYC* was a transcriptional regulator of five genes (*CCND1, AZGP1, PTP4A3, MYBL1, and RNF130*) ($p=3.1\times10^{-4}$).

Discussion

To characterize genes and molecular pathways influencing survival across myeloma subtypes, we assessed expression levels of over 55,000 gene probes from tumor cells obtained from 1,188 newly diagnosed myeloma patients. 71 genes were significantly altered in patients with the *MMSET* molecular subtype. Selecting from these genes, 10-gene risk score demonstrated similar 5-year survivals between *MMSET* myeloma and Non-*MMSET* patients categorized as the top quartile risk score (*MMSET*-like myeloma). A 5-fold cross-validation was conducted to determine the unbiased association of the risk score and survival. Pathway analysis identified *MYC* and *TP53* transcriptional regulators were associated with the observed gene-expresssion changes of 10 genes.

Of clinical relevance, our findings suggest an overlap of disease biology between conventionally divided groups of high-risk and non-high-risk myelomas. The study findings should be interpreted within the context of recent advancement of genetic sequencing, which refined tumor subtypes based on recurrent genetic alterations. In ovarian cancer, approximately half of the patients were found to have homologous recombination deficiency (HRD) mimicking the genetic phenotype of *BRCA* mutation (i.e. “BRCA-ness”). (20) Intriguingly, the presence of HRD in *BRCA* wild-type patients predicted striking sensitivity to PARP inhibition in a prospective trial (overall response rate: 32% with HRD vs. 11% without HRD), albeit less than the true *BRCA* mutated group (66%). Evolving knowledge in biological homology across different tumor subtypes proposes a new therapeutic strategy is required to improve the outcome of patients with *MMSET*-like gene signature. As seen in differential responsiveness to PARP inhibition in cancers with *BRCA*-ness, *MMSET*-like subgroup may also benefit from established or investigational regimens developed for high-risk myeloma, rather than those developed for standard-risk population. Such regimens tested in high-risk myeloma include proteasome inhibitors (21-23) and other investigational agents aimed at novel targets such as FGFR3, (5) CD38, (24) and MEK pathway. (25) Further research needs to validate the role of genomic risk stratification tools to capture high-risk population, and to prospectively assess clinical outcome to potential treatment options within the identified subgroup.

Another important observation of this study is the demonstration of *TP53* and *MYC* as downstream targets of *MMSET* gene signature. t(4;14) accounts for 15% of myeloma population and is linked to universal overexpression of *MMSET* gene. (3, 4) Histone methyltransferase encoded at catalytic SET domain methylates lysine residue of histone,

leading to epigenetic regulation of genes involved in cell cycle progression, p53 pathway, and integrin signaling. (7) The role of *MMSET* as a myeloma oncogene is supported by an experimental knock-down of *MMSET* in myeloma cell-lines, which led to decreased proliferation and increased apoptosis. (2, 26, 27), Among many targets altered by *MMSET* overexpression, c-myc is an important downstream pathway enhanced by *MMSET* through down-regulation of miR-126. (28, 29) The overlap of p53 and *MYC* pathways has also been described in model systems of other malignancies. *In vitro*, p53 represses c-myc transcription by deacetylation of histone located at c-myc promoter (30) and by miR-145-mediated gene silencing, (31) and arrests cell cycle. These findings support the primary role of *MMSET* as a regulator of epigenetic machineries, rather than genetic instability, and is corroborated by findings from whole exome sequencing which demonstrated only a few mutational changes in the t(4;14) subgroup. (32) Taken together, an aggressive clinical phenotype of *MMSET* overexpression is attributable to the fine-tuning of selected genes. Functional studies are required to assess direct binding or indirect modulation of 10 genes by *MSMET* and to validate downstream activity of *MMSET*-like signature converging into selected signaling pathways, such as *MYC* and p53.

Gene-expression profiling is a mature and robust technology with many validated platforms in multiple myeloma reported to date. (33) Compared to previously established platforms, *MMSET*-like signature has several unique aspects. *First, MMSET*-like gene signature was developed from a biologically homogeneous population with a single genetically defined abnormality, and was applied to the overall population with an aim to select patients influenced by similar pathobiology. This sequence of development is reversed from what had been done in conventional studies, which performed hierarchical gene clustering among biologically heterogeneous population. (34) By using the latter method, a given gene-expression group can contain several different genetic abnormalities within the subtype, (6) which may have led to inconsistent results in predicting therapeutic responses. (35) The 10-gene signature proposed by the current study was developed from a homogenous subgroup, hence may be more representative of a single biological entity and can serve a useful risk stratification tool for treatment trials. *Second,* with the exception of one gene, 10-gene signature did not overlap with previously reported platforms such as EMC 92-gene, (34) UAMS 70-gene (6) and IFM 15-gene signatures. (36) This finding further supports that *MMSET*-like gene signature represents a distinct biological subtype utilizing a selected set of genes. Interestingly, *ROBO1* was the only gene within our 10-gene platform that was previously reported in another gene expression profile (37) and in a sequencing study as a candidate gene in myeloma. (32) Downstream of *ROBO1* is associated with E-cadherin mediated regulation of WNT signaling in pancreatic cancer, and its functional role in myeloma remains to be studied.

We demonstrated 10-gene signature that were significantly altered in *MMSET* myeloma and associated with inferior survival in Non-*MMSET* myeloma patients. Pathway analysis of the *MMSET*-like gene signature recapitulated clustering of important signaling pathways in myeloma, specifically *TP53* and *MYC* pathways. *MMSET*-like gene expression profile was able to capture a distinct biological subtype under-represented by conventional platforms, and was strongly linked poor clinical outcome. The proposed gene signature can serve as a

reliable screening platform representative of high-risk disease biology, as we move towards personalized therapy for myeloma.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

None

Grant Support: This work was supported by the intramural research program of the National Institutes of Health. Research support for S.P.W. was made possible through the National Institutes of Health (NIH) Medical Research Scholars Program, a public-private partnership supported jointly by the NIH and generous contributions to the Foundation for the NIH from Pfizer Inc, The Leona M. and Harry B. Helmsley Charitable Trust, and the Howard Hughes Medical Institute, as well as other private donors. For a complete list, please visit the Foundation website at http://www.fnih.org/work/programs-development/medical-research-scholars-program).

References

1. Cavo M, Terragna C, Renzulli M, Zamagni E, Tosi P, Testoni N, et al. Poor outcome with front-line autologous transplantation in t(4;14) multiple myeloma: low complete remission rate and short duration of remission. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2006; 24:e4–5. [PubMed: 16421414]
2. Chesi M, Nardini E, Lim RS, Smith KD, Kuehl WM, Bergsagel PL. The t(4;14) translocation in myeloma dysregulates both FGFR3 and a novel gene, MMSET, resulting in IgH/MMSET hybrid transcripts. Blood. 1998; 92:3025–34. [PubMed: 9787135]
3. Santra M, Zhan F, Tian E, Barlogie B, Shaughnessy J Jr. A subset of multiple myeloma harboring the t(4;14)(p16;q32) translocation lacks FGFR3 expression but maintains an IGH/MMSET fusion transcript. Blood. 2003; 101:2374–6. [PubMed: 12433679]
4. Keats JJ, Maxwell CA, Taylor BJ, Hendzel MJ, Chesi M, Bergsagel PL, et al. Overexpression of transcripts originating from the MMSET locus characterizes all t(4;14)(p16;q32)-positive multiple myeloma patients. Blood. 2005; 105:4060–9. [PubMed: 15677557]
5. Mirabella F, Wu P, Wardell CP, Kaiser MF, Walker BA, Johnson DC, et al. MMSET is the key molecular target in t(4;14) myeloma. Blood Cancer J. 2013; 3:e114. [PubMed: 23645128]
6. Shaughnessy JD Jr, Zhan F, Burington BE, Huang Y, Colla S, Hanamura I, et al. A validated gene expression model of high-risk multiple myeloma is defined by deregulated expression of genes mapping to chromosome 1. Blood. 2007; 109:2276–84. [PubMed: 17105813]
7. Martinez-Garcia E, Popovic R, Min DJ, Sweet SM, Thomas PM, Zamdborg L, et al. The MMSET histone methyl transferase switches global histone methylation and alters gene expression in t(4;14) multiple myeloma cells. Blood. 2011; 117:211–20. [PubMed: 20974671]
8. Marsit CJ, Liu M, Nelson HH, Posner M, Suzuki M, Kelsey KT. Inactivation of the Fanconi anemia/BRCA pathway in lung and oral cancers: implications for treatment and survival. Oncogene. 2004; 23:1000–4. [PubMed: 14647419]
9. Antoniou AC, Casadei S, Heikkinen T, Barrowdale D, Pylkas K, Roberts J, et al. Breast-cancer risk in families with mutations in PALB2. The New England journal of medicine. 2014; 371:497–506. [PubMed: 25099575]
10. Weischer M, Nordestgaard BG, Pharoah P, Bolla MK, Nevanlinna H, Van't Veer LJ, et al. CHEK2*1100delC heterozygosity in women with breast cancer associated with early death, breast cancer-specific death, and increased risk of a second breast cancer. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2012; 30:4308–16. [PubMed: 23109706]
11. Easton DF, Pharoah PD, Antoniou AC, Tischkowitz M, Tavtigian SV, Nathanson KL, et al. Gene-panel sequencing and the prediction of breast-cancer risk. The New England journal of medicine. 2015; 372:2243–57. [PubMed: 26014596]

12. Multiple Myeloma Genome Portal. [cited; Available from: http://www.broadinstitute.org/mmgp
13. Zhan F, Huang Y, Colla S, Stewart JP, Hanamura I, Gupta S, et al. The molecular classification of multiple myeloma. Blood. 2006; 108:2020–8. [PubMed: 16728703]
14. Dimopoulos M, Gika D, Zervas K, Kyrtsonis M, Symeonidis A, Anagnostopoulos A, et al. The international staging system for multiple myeloma is applicable in symptomatic Waldenstrom's macroglobulinemia. Leuk Lymphoma. 2004; 45:1809–13. [PubMed: 15223640]
15. Pfeiffer RM, Gail MH, Pee D. On combining data from genome-wide association studies to discover disease-associated SNPs. Statistical Science. 2009; 24:547–60.
16. Zangari M, van Rhee F, Anaissie E, Pineda-Roman M, Haessler J, Crowley J, et al. Eight-year median survival in multiple myeloma after total therapy 2: roles of thalidomide and consolidation chemotherapy in the context of total therapy 1. Br J Haematol. 2008; 141:433–44. [PubMed: 18371114]
17. Morgan GJ, Gregory WM, Davies FE, Bell SE, Szubert AJ, Brown JM, et al. The role of maintenance thalidomide therapy in multiple myeloma: MRC Myeloma IX results and meta-analysis. Blood. 2012; 119:7–15. [PubMed: 22021371]
18. Molinaro AM, Simon R, Pfeiffer RM. Prediction error estimation: a comparison of resampling methods. Bioinformatics. 2005; 21:3301–7. [PubMed: 15905277]
19. Molecular Signaling Database from the Broad Institute. [cited; Available from: http://www.broadinstitute.org/gsea/msigdb
20. McNeish I, Oza A, Coleman RL, Scott CL, Konecny G, O'Malley DM, et al. Results of ARIEL2: A Phase 2 trial to prospectively identify ovarian cancer patients likely to respond to rucaparib using tumor genetic analysis. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2015; 33 (abstract 5508).
21. Avet-Loiseau H, Leleu X, Roussel M, Moreau P, Guerin-Charbonnel C, Caillot D, et al. Bortezomib plus dexamethasone induction improves outcome of patients with t(4;14) myeloma but not outcome of patients with del(17p). Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2010; 28:4630–4. [PubMed: 20644101]
22. Neben K, Lokhorst HM, Jauch A, Bertsch U, Hielscher T, van der Holt B, et al. Administration of bortezomib before and after autologous stem cell transplantation improves outcome in multiple myeloma patients with deletion 17p. Blood. 2012; 119:940–8. [PubMed: 22160383]
23. Jakubowiak AJ, Siegel DS, Martin T, Wang M, Vij R, Lonial S, et al. Treatment outcomes in patients with relapsed and refractory multiple myeloma and high-risk cytogenetics receiving single-agent carfilzomib in the PX-171-003-A1 study. Leukemia. 2013; 27:2351–6. [PubMed: 23670297]
24. Lokhorst HM, Plesner T, Laubach JP, Nahi H, Gimsing P, Hansson M, et al. Targeting CD38 with Daratumumab Monotherapy in Multiple Myeloma. N Engl J Med. 2015; 373:1207–19. [PubMed: 26308596]
25. Holkova B, Zingone A, Kmieciak M, Bose P, Badros AZ, Voorhees PM, et al. A Phase II Trial of AZD6244 (Selumetinib, ARRY-142886), an Oral MEK1/2 Inhibitor, in Relapsed/Refractory Multiple Myeloma. Clin Cancer Res. 2015 [Epub ahead of print].
26. Lauring J, Abukhdeir AM, Konishi H, Garay JP, Gustin JP, Wang Q, et al. The multiple myeloma associated MMSET gene contributes to cellular adhesion, clonogenic growth, and tumorigenicity. Blood. 2008; 111:856–64. [PubMed: 17942756]
27. Brito JL, Walker B, Jenner M, Dickens NJ, Brown NJ, Ross FM, et al. MMSET deregulation affects cell cycle progression and adhesion regulons in t(4;14) myeloma plasma cells. Haematologica. 2009; 94:78–86. [PubMed: 19059936]
28. Min DJ, Ezponda T, Kim MK, Will CM, Martinez-Garcia E, Popovic R, et al. MMSET stimulates myeloma cell growth through microRNA-mediated modulation of c-MYC. Leukemia. 2013; 27:686–94. [PubMed: 22972034]
29. Popovic R, Martinez-Garcia E, Giannopoulou EG, Zhang Q, Zhang Q, Ezponda T, et al. Histone methyltransferase MMSET/NSD2 alters EZH2 binding and reprograms the myeloma epigenome through global and focal changes in H3K36 and H3K27 methylation. PLoS Genet. 2014; 10:e1004566. [PubMed: 25188243]

30. Ho JS, Ma W, Mao DY, Benchimol S. p53-Dependent transcriptional repression of c-myc is required for G1 cell cycle arrest. Mol Cell Biol. 2005; 25:7423–31. [PubMed: 16107691]
31. Sachdeva M, Zhu S, Wu F, Wu H, Walia V, Kumar S, et al. p53 represses c-Myc through induction of the tumor suppressor miR-145. Proc Natl Acad Sci U S A. 2009; 106:3207–12. [PubMed: 19202062]
32. Bolli N, Avet-Loiseau H, Wedge DC, Van Loo P, Alexandrov LB, Martincorena I, et al. Heterogeneity of genomic evolution and mutational profiles in multiple myeloma. Nat Commun. 2014; 5:2997. [PubMed: 24429703]
33. Chng WJ, Dispenzieri A, Chim CS, Fonseca R, Goldschmidt H, Lentzsch S, et al. IMWG consensus on risk stratification in multiple myeloma. Leukemia. 2014; 28:269–77. [PubMed: 23974982]
34. Kuiper R, Broyl A, de Knegt Y, van Vliet MH, van Beers EH, van der Holt B, et al. A gene expression signature for high-risk multiple myeloma. Leukemia. 2012; 26:2406–13. [PubMed: 22722715]
35. Amin SB, Yip WK, Minvielle S, Broyl A, Li Y, Hanlon B, et al. Gene expression profile alone is inadequate in predicting complete response in multiple myeloma. Leukemia. 2014; 28:2229–34. [PubMed: 24732597]
36. Decaux O, Lode L, Magrangeas F, Charbonnel C, Gouraud W, Jezequel P, et al. Prediction of survival in multiple myeloma based on gene expression profiles reveals cell cycle and chromosomal instability signatures in high-risk patients and hyperdiploid signatures in low-risk patients: a study of the Intergroupe Francophone du Myelome. J Clin Oncol. 2008; 26:4798–805. [PubMed: 18591550]
37. Zhan F, Hardin J, Kordsmeier B, Bumm K, Zheng M, Tian E, et al. Global gene expression profiling of multiple myeloma, monoclonal gammopathy of undetermined significance, and normal bone marrow plasma cells. Blood. 2002; 99:1745–57. [PubMed: 11861292]

Statement of Translational Relevance

Multiple myeloma is a biologically and clinically heterogeneous disease. The presence of biological homology shared between conventional high-risk and non-high-risk myeloma subgroups has not been reported to date. We hypothesized that molecular risk stratification can capture biological homology between patients with or without *MMSET* overexpression, and be used as a prognostic tool. We identified 10-gene signature associated with *MMSET* myeloma. We obtained gene-expression profiles of 1,032 newly diagnosed myeloma patients enrolled in Total Therapy 2, Total Therapy 3, Myeloma IX, and HOVON65-GMMGHD4 trials, and 156 patients from Multiple Myeloma Resource Collection. Expression of *MMSET*-like gene signature in Non-*MMSET* subgroup was associated with similarly poor survival. Pathway analysis of *MMSET*-like gene signature revealed the involvement of p53 and *MYC* signaling pathways. *MMSET*-like gene signature captures a subset of high-risk myeloma patients under-represented by conventional risk stratification platforms, and defines a distinct biological subtype.

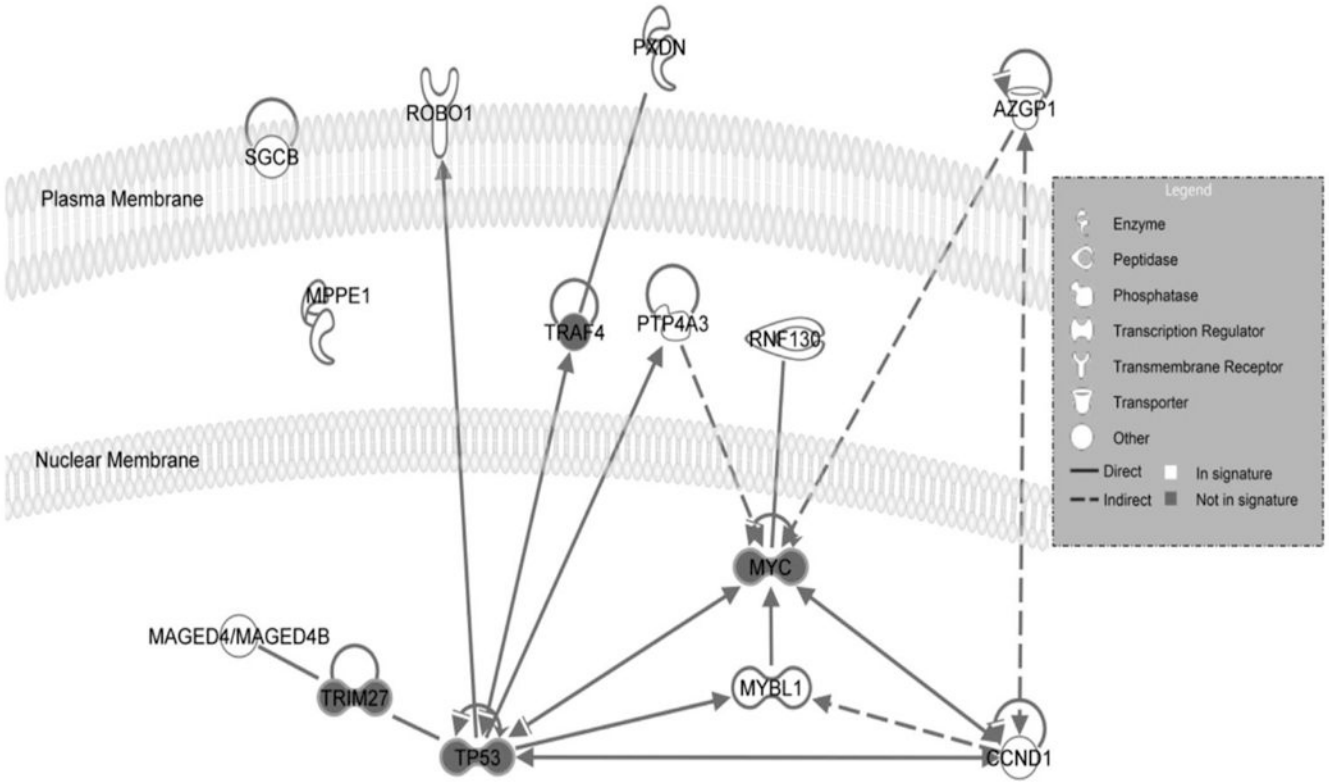

Figure 1.

Full lines represent direct interactions while dashed lines indicate indirect interactions. An arrow pointing from one protein to another indicates that the first protein acts on or activates the second protein (at which the arrow is pointing).

Table 1

Patient and study characteristics

	Induction Therapy	Maintenance	Median Age, Years (range)	Women, N (%)	ISS 1, N (%)	ISS 2, N (%)	ISS 3, N (%)	Maximum Follow-up (years)
TT2 (N=345) TT3 (N=208)	D(T)-PACE (N=345)	Thalidomide	57 (24-77)	148 (43)	184 (53)	90 (26)	71 (21)	8.2
	VTD-PACE (N=208)	Bort-Thal-Dex	60 (32-75)	72 (34)	100 (48)	64 (31)	44 (21)	4.4
HOVON65/GMMGHD5 (N=296)	VAD (N=143)	Thalidomide	58 (27-65)	61 (43)	55 (38)	44 (31)	44 (31)	6.1
	PAD (N=153)	Bortezomib	56 (31-65)	58 (38)	51 (33)	62 (41)	40 (26)	5.8
Myeloma IX (N=183)	CTD (N=58)	(+/-) Thalidomide (N=30)	59.5 (45-69)	11 (37)	8 (27)	12 (40)	10 (33)	8.1
		NULL (28)	61 (35-68)	13 (46)	10 (36)	10 (36)	8 (28)	7.6
	CVAD (N=48)	(+/-) Thalidomide (N=23)	57 (39-69)	6 (26)	8 (35)	7 (30)	8 (35)	7.6
		NULL (N=25)	60 (48-68)	10 (40)	6 (24)	9 (36)	10 (40)	7.4
	CTDa (N=41)	(+/-) Thalidomide (N=19)	73 (67-83)	8 (42)	2 (11)	5 (27)	12 (63)	7.5
		NULL (N=22)	73.5 (61-84)	12 (55)	1 (5)	11 (50)	10 (46)	7.7
	Melphalan (N=36)	(+/-) Thalidomide (N=15)	70 (63-80)	8 (53)	5 (33)	5 (33)	5 (33)	7.2
		NULL (N=21)	74 (62-89)	8 (38)	2 (10)	7 (34)	12 (57)	6.5
MMRC [*] (N=156)	-[*]	-[*]	60 (24-89)	51 (32)	74 (48)	46 (30)	36 (23)	-[*]
All Studies (N=1188)	-[*]	-[*]	**59 (24-89)**	**466 (39%)**	**506 (43%)**	**372 (31%)**	**310 (26%)**	**8.2**

D(T)-PACE: Dexamethasone with or without thalidomide, cisplatin P, doxorubicin A, cyclophosphamide C, etoposide E. VTD-PACE: V Bortezomib. PAD: Bortezomib P, Doxorubicin A, Dexamethasone D, VAD: Vincristine V, Doxorubicin A, Dexamethasone D. CTD/CVAD: Cyclophosphamide C, Thalidomide T, Doxorubicin A, Dexamethasone D.

[*] =information not available

Table 2

Genes identified as highly correlated to *MMSET* myeloma and associated with survival in Non-*MMSET* patients

gene	probe	Lin Reg coef[1]	p-value	HR[2] (95% CI)	p-value	TP53 Interacting	MYC Interacting	annotation
CCND1	208712_at	-3.6	1.70E-33	0.90 (0.86-0.94)	<.0001	**Yes**	**Yes**	cyclin D1
AZGP1	209309_at	3.08	6.40E-25	1.10 (1.05-1.16)	<.0001	No	**Yes**	alpha-2-glycoprotein 1 zinc
SGCB	205120_s_at	2.51	3.90E-17	1.09 (1.03-1.15)	0.0014	No	No	sarcoglycan beta (dystrophin-associated glycoprotein)
MAGED4	223313_s_at	2.95	5.00E-23	0.92 (0.87-0.97)	0.0033	No	No	melanoma antigen family D 4
PXDN	212012_at	2.21	1.30E-13	1.09 (1.03-1.15)	0.0035	No	No	peroxidasin homolog (Drosophila)
RNF130	217865_at	2.47	1.40E-16	0.93 (0.89-0.98)	0.0053	No	**Yes**	ring finger protein 130
MYBL1	213906_at	2.5	5.30E-17	1.08 (1.02-1.14)	0.0086	**Yes**	**Yes**	v-myb myeloblastosis viral oncogene homolog (avian)-like 1
PTP4A3	206574_s_at	2.51	4.70E-17	0.94 (0.90-0.99)	0.0104	**Yes**	**Yes**	protein tyrosine phosphatase type IVA member 3
MPPE1	213924_at	2.28	2.10E-14	0.92 (0.86-0.98)	0.0109	No	No	Metallophosphoesterase 1
ROBO1	213194_at	2.23	8.60E-14	1.06 (1.01-1.11)	0.0288	**Yes**	No	roundabout axon guidance receptor homolog 1 (Drosophila)

[1] linear regression coefficient associated with MMSET from meta analysis that uses log2 transformed probe levels as outcome

[2] HRs from adjusted Cox regression model that fit each probe separately to 5-year survival